Polymorphisms in placental iodothyronine deiodinase genes are not associated with neural tube defects in pregnant women with high maternal serum homocysteine and low thyroid hormone levels

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Abstract

Background: This study hypothesized that single nucleotide polymorphisms in placental iodothyronine deiodinase genes could be related to neural tube defects in pregnant women with high maternal serum homocysteine and low thyroid hormone levels.

Methods: We performed a case-control study between 2007 and 2009 that included pregnant women from the Lüliang Mountains, Shanxi Province, China. Nine distinct single nucleotide polymorphisms in the iodothyronine deiodinase type 1, type 2, and type 3 genes were analyzed using placental samples obtained from 83 pregnant women with fetuses harboring neural tube defects (cases) and 90 pregnant women with fetuses without neural tube defects (controls). The nine single nucleotide polymorphisms were analyzed using the Cochran–Armitage test and the Chi-squared test (Fisher's exact test).

Results: There were no statistically significant associations between the nine placental single nucleotide polymorphisms and neural tube defects ($P>0.05$). Additionally, no statistically significant relationships were found between the single nucleotide polymorphisms in placental iodothyronine deiodinase genes and neural tube defects among pregnant women with high maternal serum homocysteine and low thyroid hormone levels.

Conclusions: Our result in this study supports our previous report, which showed dysregulation of iodothyronine deiodinase type 3 transcription due to histone modifications of the promotor region in a mouse model of spina bifida. Our result also suggests that other factors such as histone modifications of the genes of deiodinases by a higher level of serum total homocysteine rather than single nucleotide polymorphisms of the genes contribute to neural tube defects in pregnant women with high maternal serum homocysteine and low thyroid hormone levels.

Introduction

Neural tube defects (NTDs) are severe birth defects of the central nervous system. A particularly high prevalence of NTDs, along with other congenital malformations, was recorded in 2003 in the Lüliang Mountains, Shanxi Province, northern China [1, 2]. In our previous studies, we found that the main causes were deficiency of folic acid and/or other micronutrients, high total homocysteine (tHcy) levels, and the coexistence of inadequate maternal serum levels of thyroid hormones, which increase the risk of elevated tHCY [3, 4, 5].

Thyroid hormones are essential for normal early fetal neurogenesis. Maternal thyroxine (T4) has been observed in embryonic circulation throughout gestation and as early as the fourth week of pregnancy [6]. Moreover, there is a peak of maternal T4 that results from high placental chorionic gonadotropin levels at approximately 10 to 12 gestational weeks [7]. The fetal thyroid gland reaches maturity by weeks 11 to 12 and begins to secrete thyroid hormones by approximately week 16 [6]. The presence of iodothyronine deiodinase type 3 (DIO3) in the placenta, uterus, and some fetal tissues is critical for minimizing the exposure of fetal tissues to inappropriate levels of thyroid hormone [8]. However, locally generated triiodothyronine (T3) in the brain from maternally transported T4 has been reported to be essential for normal early brain development [6, 9]. Almost 80% of neural T3 is produced locally by iodothyronine deiodinase type 2 (DIO2) [10]. The iodothyronine deiodinases (type 1, type 2, and type 3 (DIO1, DIO2, and DIO3, respectively)) constitute a potent mechanism of thyroid hormone activation (DIO1 and DIO2) or inactivation (DIO3). The predominant deiodinase expressed in placenta is DIO3; however, DIO2 is also present [11].

Therefore, we hypothesized that single nucleotide polymorphisms (SNPs) in the placental *DIO1*, *DIO2*, and *DIO3* genes may be associated with fetal NTDs if they occur together with high maternal serum tHCY levels and low thyroid hormone levels. This study was performed to test this hypothesis.

Methods

Study design

We performed a case-control study between 2007 and 2009 that included pregnant women from the Lüliang Mountains, Shanxi Province, China, as previously reported [5].

Participants, recruitment, and diagnostic criteria of NTDs (outcome measures)

NTDs in fetuses were diagnosed by B-mode ultrasound, which was conducted as part of a routine health checkup at or from 12 gestational weeks in several local county hospitals. When NTDs were diagnosed by B-mode ultrasound, medical abortions were performed at the local county hospitals. Medical staff collected clinical information including gestational age in weeks and serum/and spot urinary samples. Fetal samples from women who underwent pregnancy termination for nonmedical reasons were also obtained from the same area and were randomly selected on the basis of gender and gestational period. Pathological confirmation of the presence/absence of NTDs was completed by experienced pathologists from the Capital Institute of Pediatrics, Beijing, China in accordance with the International Classification of Disease, Tenth Revision, codes Q00.0, Q05.9, and Q01.9 (https://www.who.int/standards/classifications/classification-of-diseases). Routine prenatal health checkups, questionnaires, and fetal autopsy reports were completed for all fetuses. All staff involved in the project were specifically trained for this project [5].

Sample collection and transportation

All placental samples used for DNA extraction were stored at −20°C in local hospitals before shipment on ice to the study laboratories. Placental tissues were obtained from test and control subjects as previously reported [12].

DNA extraction

Genomic DNA were extracted from frozen placental tissues using a Blood and Tissue DNA Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. DNA purity was assessed by measuring light absorbance at 260 nm and 280 nm. Samples with an A260/280 ratio outside of 1.80 to 1.95 were not used. DNA concentrations were assessed by measuring the absorbance at 260 nm.

SNP assays for DIO1, DIO2, and DIO3

Nine maternal SNPs were analyzed. We analyzed rs11206237 (A/C) and rs2235544 (A/C), which are located in *DIO1*; rs12885300 (A/G), rs1388378 (A/C), rs225010 (A/G), rs225011 (C/T), rs225012 (T/C), and rs225014 (A/G), which are located in *DIO2*; and rs17716499 (C/T), which is located in *DIO3*. IPLEX Gold SNP genotyping analysis was performed to determine the SNPs in *DIO1*, *DIO2*, and *DIO3*, and the genotypes using the Sequenom MassARRAY System (CapitalBio, Beijing, China) as previously reported [5]. This system uses allele-specific extension in combination with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. PCR and extension primers were designed using RealSNP (https://www.mysequenom.com/), which specifies three primers for each SNP in the assay as follows: two primers for PCR amplification and one primer for an extension reaction *via* hybridization adjacent to the SNP site (Table 1).

Statistical analysis

Associations between SNPs and NTDs were determined by the Cochran–Armitage trend test and the Chi-squared test (Fisher's exact test) using JMP version 15 (SAS Institute Inc., Cary, NC, USA). Differences were considered statistically significant at $P<0.05$.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Board of the Capital Institute of Pediatrics, Beijing, China (cited March 8, 2004), Teikyo University, Japan (Tei-I-Rin 14-010), and Osaka Medical College, Japan (2758-01). All participants provided written informed consent.

Results

In this study, we analyzed *DIO1*, *DIO2*, and *DIO3* from frozen placental samples using SNP assays. In our previous study, we found that pregnant women with high levels of serum homocysteine and low thyroid hormone levels were associated with NTDs. In this study, we analyzed nine SNPs of *DIO1*, *DIO2*, and *DIO3* using placental samples. However, no statistically significant relationships between the SNPs and NTDs were found by analysis with the Cochran–Armitage test for trends or the Chi-squared test (Fisher's exact test) ($P>0.05$) (Table 2).

Discussion

In this study, we found no statistically significant relationships between placental SNPs of *DIO1*, *DIO2*, and *DIO3* and NTDs. Moreover, although our previous report showed a relationship between NTDs and pregnant women with high serum homocysteine levels and low thyroid hormone levels [5], this study showed that maternal SNPs of the DIO genes are not statistically related to NTDs in this population. Therefore, the results of this study suggest that high serum homocysteine levels and low thyroid hormone levels are risk factors for fetal NTDs and not placental utilization of maternal thyroid hormones, which would be influenced by SNPs of the DIO genes.

The fetus depends on maternal thyroid hormones, particularly before the onset of endogenous fetal thyroid hormone production at approximately 14 to 16 weeks of gestation [7]. The placenta is known to be a rich source of DIOs [8, 9]. DIO1 catalyzes the monodeiodination of T4 to T3. DIO2 is the primary activating enzyme that locally catalyzes the monodeiodination of T4 to T3. The highest activities of DIO2 have been reported in the central nervous system. Locally generated T3 in the fetal brain from maternally transported T4 has been reported to be essential for normal early brain development [6, 10]. DIO3 is the deactivating enzyme that catalyzes the monodeiodination of T4 to reverse T3 and of T3 to T2. DIO3 is primarily present in the placenta and to a lesser extent in the central nervous system [6,11]. Despite the relatively high DIO3 activity in the placenta, physiologically important levels of maternal T4 are transferred to the fetus [13, 14]. DIO3 plays an essential role in regulating thyroid hormone inactivation during embryonic development. Altered levels of transplacental thyroid hormone passage have an effect on neurological development. However, in this study, placental SNPs of DIO genes were not associated with NTDs, supporting our previous results that suggested that the coexistence of high levels of maternal tHCY and low levels of thyroid hormones was associated with NTDs rather than placental utility of maternal thyroid hormones, which would be influenced by SNPs of DIO genes.

As previously reported, low levels of maternal thyroid hormones are involved in folic acid metabolism by influencing maternal riboflavin metabolism, oxidative stress, and the vitamin D biosynthetic pathway [5] (Figure 1 [15, 16, 17, 18]). Therefore, abnormal levels of maternal thyroid hormones may alter fetal metabolism of folic acid. Moreover, our previous case study [19] showed that (1) in human fetuses with spinal NTDs there is possible abnormal crosstalk between thyroid hormones and retinoic acid signaling through their common retinoid X receptors and the subsequent recruitment of histone modifications; and (2) human fetuses with spinal NTDs in the context of maternal serum hyperthyroidism present elevated thyroid hormone signaling along with T3 degradation (attenuated DIO2/DIO3 switching). Therefore, abnormal levels of maternal thyroid hormones may be involved in fetal metabolism of folic acid by influencing various maternal and fetal metabolic and biosynthetic pathways (Figure 1).

A limitation of this study was the small number of subjects because (1) NTDs are a rare disease; and (2) the location of this study was in a rural area that is not conveniently located. There was no refrigerated cargo transportation at that time, and the frozen samples were transported on plenty of ice which taken places of the car. The results of this study should be verified using a large number of cases and controls in future studies.

Conclusion

Folic acid deficiency and high serum tHCY levels are well-known maternal nutrition factors that are associated with NTDs. However, the mechanism through which folic acid and multivitamin supplements prevent NTDs remains unclear. This study revealed that thyroid hormones regulate the relationship between one-carbon metabolism and other metabolic signaling pathways that are involved in NTDs.

Abbreviations

NTD: neural tube defect; tHcy total homocysteine; SNP: Single Nucleotide Polymorphism; DIO1: iodothyronine deiodinase type 1; DIO2: iodothyronine deiodinase type 2; DIO3: iodothyronine deiodinase type 3; T4: thyroxine; T3: triiodothyronine.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Board of the Capital Institute of Pediatrics, Beijing, China (cited March 8, 2004), Teikyo University, Japan (Tei-I-Rin 14-010), and Osaka Medical College, Japan (2758-01). All participants provided written informed consent.

Consent for publication

Not applicable.

Availability of data and material

Please contact author for data requests.

Competing interests

The authors declare no potential conflicts of competing interest with respect to the research, authorship, and/or publication of this article.

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Authors' contributions

WF carried out the field research, questionnaire, samples transport, and wrote original draft preparation.

GYH did hypothesis, design, formal analysis, and wrote original draft preparation.

GJ, BYH and QZY participated field research, informed consent, data collection and/or laboratory tests.

ZP cleaned data and did formal analysis.

UM & MM did formal statistical analysis.

XXH & ZT performed supervision, project administration, and funding acquisition.

All authors read and approved the final manuscript.

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Tables

Table 1. Primers for SNP genotyping on DIO1, DIO2 and DIO3 genes

Gene	Location	SNPs	Amplification primers	Extension primers
DIO1	Upstream	rs11206237	ACGTTGGATGACTTTCTGCTTCGAGGCTTG; ACGTTGGATGTTCTGGGATGCACCTGTCTG	CTGTGTGCTGATTGGCTCCAGCAAG
DIO1	Intronic region	rs2235544	ACGTTGGATGACCTCTTGCAACTAACCTCC; ACGTTGGATGCCCTGCAAGAGAAACGAATC	TCAGGCATTCCCAACTT
DIO2	Intron	rs225010	ACGTTGGATGAACCCTGACCTGCAATGAAC; ACGTTGGATGGGCCCCAGGATTCATATTTC	AACATAATCATATTTGGGTGA
DIO2	Intron	rs225011	ACGTTGGATGGGTCAGGGTTATCATTAAGTG; ACGTTGGATGATTAGGGTTAGACTGGGAAG	CGAATGAATGCTAGTTGTTATAATC
DIO2	Intron	rs225012	ACGTTGGATGCCCCACAATTTGTTAACAAGC; ACGTTGGATGGCAAAGGGAGCACATGAAAC	ACATGAAACAATTTTTATCTCTAC
DIO2	Nonsynonymous	rs225014	ACGTTGGATGTCTTCTCCTGGGTACCATTG; ACGTTGGATGATTCCAGTGTGGTGCATGTC	CTTTTGGTGCATGTCTCCAGT
DIO2	5 prime UTR	rs12885300	ACGTTGGATGTTCATTTCCAAGCACCTATG; ACGTTGGATGCAAGAAAGAAACAGGCTACG	GGGAGCATAGAGACAATGAAAG
DIO2	Intron	rs1388378	ACGTTGGATGTCCAGAGACGTTGTAAGAGG; ACGTTGGATGGGGTAGCCTCAAAATAATGC	GGCATCACACTATTTCATAA
DIO3	Downstream	rs17716499	ACGTTGGATGCAGATGGTTTAGCCTTGAGC; ACGTTGGATGAGCCACCTGTGCCTTCGAG	TAGAGTTCATAGAAAGGGTCT

SNP: single nucleotide polymorphism; DIO1: iodothyronine deiodinase 1; DIO2: iodothyronine deiodinase 2; DIO3: iodothyronine deiodinase 3; UTR: untranslated region.

Table 2. Cochran–Armitage trend test* and chi-squared (Fisher's exact test) results among placental samples

	Cases N, %	Controls N, %	P value (one-tailed)
SNPs in DIO1 gene			
rs11206237			
AA	0, 0.0	1, 3.3	0.328*
AC	10, 32.3	6, 20.0	
CC	21, 67.7	23, 76.7	
AA □ AC	10, 32.3	7, 23.3	0.437
CC	21, 67.7	23, 76.7	
AA	0, 0.0	1, 3.3	(0.492)
AC □ CC	31, 100.0	29, 96.7	
rs2235544			
AA	8, 25.8	6, 20.0	0.498*
AC	14, 45.2	17, 56.7	
CC	9, 29.0	7, 23.3	
AA □ AC	22, 71.0	23, 76.7	0.613
CC	9, 29.0	7, 23.3	
AA	8, 25.8	6, 20.0	0.590
AC □ CC	23, 74.2	24, 80.0	
SNPs located in DIO2 gene			
rs225010			
CC	0, 0.0	1, 3.3	0.276*
CT	18, 58.1	13, 43.3	
TT	13, 41.9	16, 53.3	
CC + CT	18, 58.1	14, 46.6	0.373
TT	13, 41.9	16, 53.3	
CC	0, 0.0	1, 3.3	(0.492)
CT + TT	31, 100.0	29, 96.6	
rs225011			

CC	13, 41.9	16, 55.2	0.238*
CT	18, 58.1	12, 41.4	
TT	0, 0.0	1, 3.4	
CC + CT	31, 100.0	28, 96.6	(0.483)
TT	0, 0.0	1, 3.4	
CC	13, 41.9	16, 55.2	0.305
CT + TT	18, 58.1	13, 44.8	
rs225012			
AA	0, 0.0	1, 3.3	0.276*
AG	18, 58.1	13, 43.3	
GG	13, 41.9	16, 53.3	
AA + AG	18, 58.1	14, 46.6	0.373
GG	13, 41.9	16, 53.3	
AA	0, 0.0	1, 3.3	(0.492)
AG + GG	31, 100.0	29, 96.6	
rs225014			
CC	2, 6.5	5, 17.2	0.156*
CT	20 ,64.5	17, 58.6	
TT	9, 29.0	7, 24.1	
CC + CT	22, 71.0	22, 75.8	0.668
TT	9, 29.0	7, 24.1	
CC	2, 6.5	5, 17.2	(0.774)
CT + TT	29, 93.5	24, 82.7	
rs12885300			
CC	20, 64.5	20, 66.7	0.470*
CT	10, 32.3	10, 33.3	
TT	1, 3.2	0, 0.0	

CC + CT	30, 96.8	30, 100.0	(1.000)
TT	1, 3.2	0, 0.0	
CC	20, 64.5	20, 66.7	0.860
CT + TT	11, 35.5	10, 33.3	
rs1388378			
AA	3, 9.7	3, 10.0	0.470*
AC	13, 41.9	12, 40.0	
CC	15, 48.4	15, 50.0	
AA AC	16, 51.6	15, 50.0	0.900
CC	15, 48.4	15, 50.0	
AA	3, 9.7	3, 10.0	1.000
AC CC	28, 90.3	27, 90.0	
SNPs located in DIO3 gene			
rs17716499			
CC	4, 13.3	4, 12.9	0.472*
CT	11, 33.3	10, 35.5	
TT	16, 53.3	16, 51.6	
CC + CT	15, 46.6	14, 48.4	0.893
TT	16, 53.3	16, 51.6	
CC	4, 13.3	4, 12.9	1.000
CT + TT	27, 86.6	26, 87.1	

Notes: SNP, single nucleotide polymorphism; DIO1, iodothyronine deiodinase type 1; DIO2, iodothyronine deiodinase type 2; DIO3, iodothyronine deiodinase type 3; N, number.

Figures

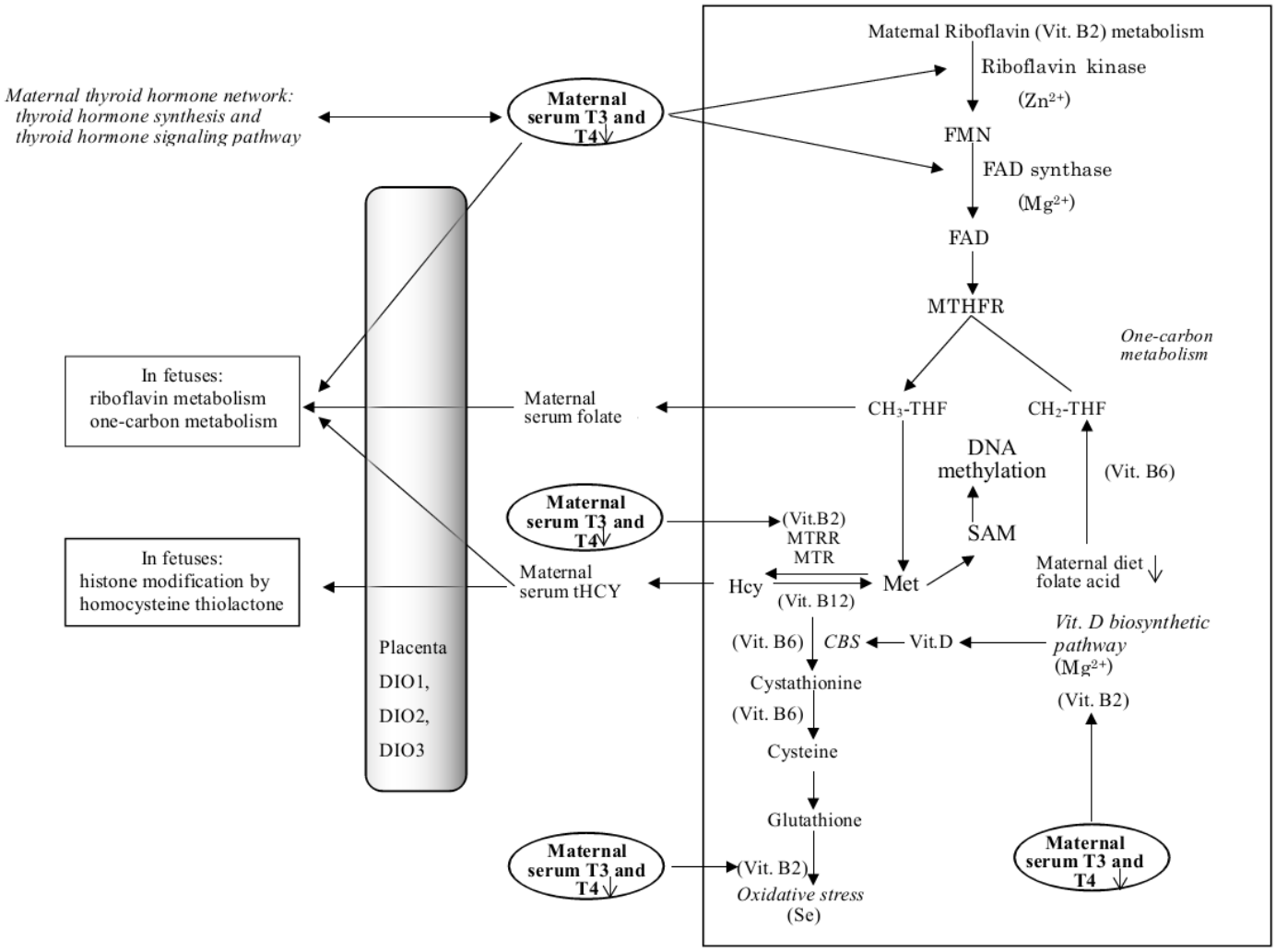

Figure 1

The placental SNPs of *DIO1*, *DIO2*, and *DIO3* that were investigated in this study and the maternal SNPs of *DIO1*, *DIO2*, and *DIO3* from our previous study (5) are not associated with NTDs, despite lower maternal serum T4 levels augmenting the risk of higher maternal serum tHCY levels, as previously reported (5). Our results suggest that although maternal thyroid hormones influence the balance between one-carbon metabolism and other metabolic signaling pathways [5, 15, 16, 17, 18] involved in NTDs, the placental SNPs of *DIO1*, *DIO2*, and *DIO3* are not associated with NTDs.

Notes: SNP, single nucleotide polymorphism; tHcy, total homocysteine; DIO1, iodothyronine deiodinase type 1; DIO2, iodothyronine deiodinase type 2; DIO3, iodothyronine deiodinase type 3; NTD, neural tube defect; SAM, S-adenosylmethionine.